In-line UV spectroscopy for the quantification of low-dose active ingredients during the manufacturing of pharmaceutical semi-solid and liquid formulations

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Abstract

UltraViolet (UV) spectroscopy was evaluated as an innovative Process Analytical Technology (PAT) - tool for the in-line and real-time quantitative determination of low-dosed active pharmaceutical ingredients (APIs) in a semi-solid (gel) and a liquid (suspension) pharmaceutical formulation during their batch production process. The performance of this new PAT-tool (i.e., UV spectroscopy) was compared with an already more established PAT-method based on Raman spectroscopy. In-line UV measurements were carried out with an immersion probe while for the Raman measurements a non-contact PhAT probe was used. For both studied formulations, an in-line API quantification model was developed and validated per spectroscopic technique. The known API concentrations (Y) were correlated with the corresponding in-line collected preprocessed spectra (X) through a Partial Least Squares (PLS) regression. Each developed quantification method was validated by calculating the accuracy profile on the basis of the validation experiments. Furthermore, the measurement uncertainty was determined based on the data generated for the determination of the accuracy profiles. From the accuracy profile of the UV- and Raman-based quantification method for the gel, it was concluded that at the target API concentration of 2 % (w/w), 95 out of 100 future routine measurements given by the Raman method will not deviate more than 10 % (relative error) from the true API concentration, whereas for the UV method the acceptance limits of 10 % were exceeded. For the liquid formulation, the Raman method was not able to quantify the API in the low-dosed suspension (0.09 % (w/w) API). In contrast, the in-line UV method was able to adequately quantify the API in the suspension. This study demonstrated that UV spectroscopy can be adopted as a novel in-line PAT-technique for low-dose

quantification purposes in pharmaceutical processes. Important is that none of the two spectroscopic techniques was superior to the other for both formulations: the Raman method was more accurate in quantifying the API in the gel (2 % (w/w) API), while the UV method performed better for API quantification in the suspension (0.09 % (w/w) API).

Keywords

In-line UV spectroscopy, In-line Raman spectroscopy, Semi-solids, Liquids, Process Analytical Technology (PAT), Accuracy profile.

1. INTRODUCTION

Spectroscopic techniques are increasingly proposed as alternative methods for the quantification of APIs in pharmaceuticals. This is due to their advantages over the traditional techniques, such as fast, in-line, non-invasive and non-destructive measurements without the need of sample preparation. Near infrared (NIR) and Raman spectroscopy have been identified as effective PAT-tools for real-time measurements of critical process and product attributes during pharmaceutical processing. Raman spectroscopy is until now mostly applied for solid dosage forms [1]–[6]. Some in-line quantitative applications for hot-melt extrusion processes have also been reported [7]–[9]. Raman spectroscopy has an added value for quantification purposes of pharmaceutical formulations where water is present, such as in semi-solid and liquid formulations, since water produces almost no Raman signal. Research has already been conducted to investigate the opportunity offered by Raman spectroscopy for these formulations [10]–[15], however less frequently as an in-line analytical tool [16]. For some applications, these spectroscopic techniques are not feasible, such as those that require the quantification of

low-dosed analytes. Fluorescence spectroscopy can be an alternative to the conventional spectroscopic techniques for these applications because of its high sensitivity and detection sensitivity [17], [18]. A drawback of fluorescence spectroscopy is that the analyte needs to be a native fluorophore in order to detect it, which limits the number of possible applications for this technique [19].

UV spectroscopy is a widely used quantitative analytical technique that finds its application in many research domains and is capable of quantifying very low concentrations (< 0.01 %) [20]–[24]. Nevertheless, studies describing on-line and in-line applications of UV/VIS spectroscopy with fibre-optic probes are limited. O'Keeffe et al. monitored the ozone concentration of a gas in an aluminium glass cell with a fibre-based UV/VIS spectroscopy system [25]. Quinn et al. followed the reaction of a nucleoside with trityl chloride in pyridine in a liquid environment [26], using a fibre-optic transmission probe. The concentration of starting material and product was predicted via a PLS regression model. Furthermore, a mixing study using a fibre-optic UV/VIS monitoring technique was reported by Ng and Assirelli [27]. In this paper, bromophenol blue sodium salt was used as a non-reactive tracer in distilled water. A good agreement between the UV/VIS technique and the traditional conductivity technique was found. Other examples of on-line and in-line UV spectroscopic applications in literature are drug dissolution tests, where the drug release was monitored in real-time [24], [28]. However, the use of UV spectroscopy for in-line monitoring of critical quality attributes during pharmaceutical manufacturing processes of semi-solids and liquids is not yet described in literature.

In this study, UV spectroscopy was evaluated as a new PAT-tool for the in-line and real-time monitoring of the API concentration during the production of pharmaceutical semi-

solid and liquid formulations. Furthermore, the performance of this new PAT-tool was compared with an already established and widely adopted PAT-method based on Raman spectroscopy. The in-line UV spectroscopic measurements were carried out by an immersion probe. For the in-line Raman measurements, a PhAT probe was used. This type of Raman probe was until now only applied in pharmaceutical unit operations such as milling, blending and coating of solid dosage forms [29]. A pharmaceutical gel and suspension with an API concentration of 2 and 0.09 % (w/w), respectively, were selected as model formulations. For both formulations, a PLS regression model was developed per spectroscopic technique and the quantification abilities of both techniques were compared. The validation of the calibration models was assessed via accuracy profiles, a validation strategy for quantitative analytical procedures proposed by the Société Francaise des Sciences et Techniques Pharmaceutiques (SFSTP) [30]–[32].

2. MATERIALS AND METHODS

2.1. Materials

Commercially available pharmaceutical formulations were kindly provided by Janssen Pharmaceutica (Beerse, Belgium): a semi-solid (gel) and a liquid (suspension), having an API target concentration of 2 % and 0.09 % (w/w), respectively. Laboratory-scale batches of the formulations were manufactured based on confidential information provided by Janssen Pharmaceutica.

2.2. Methods

2.2.1. Experimental setup

All formulations were produced with a customized IKA LR2000 mixing system (IKA, Staufen, Germany). The mixing vessel was equipped with a heated jacket for controlling the temperature of the process using a water bath (Type 1032, GFL, Burgwedel, Germany). Interface openings were provided in the cover of the mixing vessel for the implementation of the UV and Raman probe (figure 1).

2.2.2. Calibration and validation samples

In total, one calibration batch and three validation batches were produced for each formulation. Validation batch one and three were produced by operator A and validation batch two by operator B. Also, the validation batches were produced on three different days. Instead of producing a complete batch for each concentration level of the calibration (80, 90, 95, 100, 105, 110 and 120 % relative to target) and validation (85, 95, 100, 105 and 115 % relative to target) set, all the concentration levels were created using one calibration batch and three validation batches (three different days). This was done by the stepwise addition of API to a batch, corresponding to the different concentration levels. The calibration batch was produced following the standard batch production procedure of the formulations. However, instead of producing a batch with the target API concentration (i.e., 100 % of target), the calibration batch contained only 80 % of the target API concentration. After completing batch manufacturing, spectra of the lowest concentration level (i.e., 80 % of target) were collected in-line while the formulation was being mixed. Next, a specific amount of API was added to the calibration batch, corresponding to the subsequent concentration level (i.e., 90 % of target), followed by the collection of spectra. These steps (i.e., API addition and spectra recording) were repeated until the highest concentration level (i.e., 120 % of target) was reached for the calibration batch, and

spectra were recorded at each concentration. The validation batches were produced following the same procedure as described for the calibration batch, but with other concentration levels (85, 95, 100, 105 and 115 % relative to target). During this procedure (i.e., API addition and spectra recording), the formulation was mixed with a constant mixing speed.

2.2.3. UV spectroscopy

An Avaspec-ULS2048L spectrometer (Avantes, Apeldoorn, The Netherlands), equipped with a CCD detector, was connected by a fibre-optic cable to an immersion probe with a 45 degree angle window. The probe contained six illumination fibres and one detection fibre. The light source was an AvaLight Deuterium-Halogen Lamp. All spectra were acquired in the 200 - 1100 nm spectral range. The exposure time was 1000 ms and 950 ms for the gel and suspension, respectively, with each spectrum the average of 5 scans and a total of 40 spectra/concentration level. The immersion probe was inserted via the cover of the mixing vessel through a custom made interface (figure 1b).

2.2.4. Raman spectroscopy

In-line Raman spectra were recorded using a Raman Rxn2 spectrometer (Kaiser Optical Systems, Ann Arbor, MI, USA), equipped with a CCD detector and a fibre-optic PhAT probe. The laser wavelength was 785 nm with a laser power of 400 mW. The spectral range of the system was 150 - 1890 cm^{-1} with a resolution of 5 cm^{-1}. For all formulations an exposure time of 15 s with no averaging was used and every 30 s a spectrum was recorded. Per concentration level, 30 spectra were collected in-line for both the calibration and validation sets. The Raman PhAT probe was implemented through an opening in the

cover of the mixing vessel and fixed with a sealing to ensure a fixed probe position (figure 1c).

2.2.5. Development of the calibration models

For each formulation one calibration model per spectroscopic technique was developed (table 1). The UV calibration model of the gel was developed applying mean-centering, Standard Normal Variate (SNV) correction and first-derivative transformation as preprocessing methods in combination with selecting the spectral region between 280 - 297 nm (table 1). The Raman spectra of the gel were mean-centered and SNV corrected, followed by taking the first derivative and selecting the spectral regions where the API showed Raman activity. SNV preprocessing was applied to eliminate baseline offset variations, which can be caused by scatter differences between the samples. First derivative transformation allowed a better visualization of small absorption bands and corrected for baseline shifts [33].

The API concentrations (Y) were regressed against the corresponding in-line collected preprocessed spectra (X) through a PLS method. The goodness of fit and the predictive ability of the developed PLS models were assessed by the calculation of R^2 and Q^2, respectively. Q^2 values were obtained after performing a leave-one-out cross-validation, in which sub-models were developed from a reduced calibration dataset and the excluded data was predicted by the sub-models. The number of PLS components providing the highest Q^2 value was selected. Details of the developed UV and Raman PLS models of the suspension are also displayed in table 1. The PLS models were created using the SIMCA software (Version 14, Umetrics, Umeå, Sweden).

2.2.6. Validation of the calibration models

The predictive properties of the developed models were first assessed by computation of the Root Mean Square Error of Prediction (RMSEP), obtained when predicting the external validation sets. During validation, the within-day, between-day and operator variability were incorporated. Accuracy profiles were adopted to evaluate the validation of the developed analytical methods and are proposed by SFSTP as a harmonized approach for the validation of quantitative analytical procedures [30]–[32]. The objective of validation is to ensure that the difference between the measured value (x_i) and the unknown true value of the sample (μ_T) will be lower than an acceptance limit (λ):

$$|x_i - \mu_T| < \lambda \quad (1)$$

Here, λ was set at 10 %. For an analytical method to be considered as acceptable, it must be assured that the probability that a measurement will fall outside the acceptance limits is less than or equal to the maximum risk that the analyst is able to take during routine use:

$$\Pr(|x_i - \mu_T| < \lambda) \geq \beta \quad (2)$$

The desired proportion of measurements inside the acceptance limits (β) was set at 95 %. The computation of a large number of validation parameters (e.g., precision, trueness, linearity, …) is not sufficient to decide whether the objectives of validation are ensured. Therefore, the accuracy profile was used as a decision tool for the validity of the analytical methods, which is constructed from the total error of the method, being the sum of the random error (precision) and systematic error (trueness) [32]. For the precision, both the

repeatability (within-day variability) and intermediate precision (between-day and operator variability) were calculated [34]. In the accuracy profiles, the acceptance limits are plotted together with the relative error of the individual predictions, the relative bias and the β-expectation tolerance intervals at each concentration level of the validation set. Here, the acceptance limits were set at 10 % relative error. The β-expectation tolerance intervals visualise at each concentration level where at least 95 out of 100 future measurements given by the analytical procedure will fall between [35]. The intersect between the acceptance limits and the β-expectation tolerance intervals defines the upper and lower quantification limits of the analytical method. The accuracy profiles were calculated from the data obtained from the validation experiments.

Furthermore, the standard deviation of the β-expectation tolerance intervals was used for the estimation of the standard uncertainty in the measurements [36]. The uncertainty is defined as a parameter associated with the result of a measurement, that characterises the dispersion of the values that could reasonably be attributed to the measurand. The measurement uncertainty was expressed by four uncertainty parameters: uncertainty of the bias, uncertainty (combination of uncertainty of the bias with the intermediate precision standard deviation), expanded uncertainty and the relative expanded uncertainty. The expanded uncertainty represents an interval around the mean value where the unknown true value can be located with a certain confidence level (here 95 %). The relative expanded uncertainty is calculated as the expanded uncertainty divided by the corresponding true concentration [37].

3. RESULTS AND DISCUSSION

The development and validation of the PLS models for the gel formulation, based on the measurements with the two spectroscopic techniques (UV and Raman spectroscopy), will be discussed in detail in the results section. Information regarding the development and validation of the PLS models of the suspension can be found in tables 1, 2, 3 and 4.

3.1. Development of the calibration models

3.1.1. UV spectroscopy

The in-line UV/VIS measurements were made in the 200 - 1100 nm spectral range. Only the UV region (200 - 400 nm) was investigated, since the size of the conjugated system of the API was not large enough to absorb in the VIS region [38]. Also, prominent deuterium peaks were present in the VIS region (486 and 656 nm), which were not of interest [39]. In a first step, the molecular structure of the API in the gel was screened for UV activity. Several aromatic groups were found in the molecular structure and suggested that the API will absorb in the UV region. The exact absorption wavelength is dependent of the type and number of functional groups coupled to the aromatic rings, which can shift the absorption wavelength to lower or higher wavelengths [38]. To confirm whether the API could indeed be detected in the UV spectra of the gel, where possible interfering components are present, the spectra of the calibration batch were coloured according to concentration level and it was checked whether the colours were in sequence with the concentration levels. A distinctive peak in the region 280 - 297 nm was observed in the SNV-corrected and first-derived UV spectra of the gel, where the spectra were clearly clustered according to API concentration (figure 2).

A PLS model was developed from the mean-centered, SNV-corrected and first-derived UV spectra of the gel between 280 - 297 nm (R^2 = 0.988; Q^2 = 0.988; Root Mean Square Error of Cross Validation (RMSECV) = 0.0274 % w/w) (table 1 and 2). Selecting this spectral region eliminated interfering variance sources, thereby increasing the variance due to concentration differences. RMSEP values (0.0584, 0.0709 and 0.0588 % w/w) of the gel were calculated from the predictions of the three validation batches. Also for the suspension a calibration model was developed, following the same strategy as described for the gel (table 1 and 2).

3.1.2. Raman spectroscopy

The Raman spectra of the gel formulation (calibration batch) and pure API are presented in figure 3. The peaks in the spectra of the pure API with the highest intensity are situated around 396, 660, 1348 and 1590 cm^{-1}. It can be noticed from figure 3 that at these Raman shifts, peaks in the spectra of the gel are visible. A detail of the preprocessed spectra of the gel calibration set at the above mentioned spectral regions is shown in figure 4. Applying these preprocessing methods highlighted the spectral differences most. A logic concentration trend in the spectra was observed at the API selective bands: increasing Raman intensity for an increasing API concentration. These four regions were the most abundant peaks in the Raman spectra of the pure API (figure 3), suggesting that the trend in the spectra was caused by the difference in API concentration.

The model of the gel formulation with the highest predictive performance (R^2 = 0.973; Q^2 = 0.973; RMSECV = 0.0418 % w/w) was created from the mean-centered, SNV corrected and first-derived Raman spectra in the regions 390 - 405, 655 - 667, 1340 - 1355 and

1570 - 1600 cm^{-1} (table 1). The selection of these spectral regions was based on the evaluation of the Raman spectra of the pure API and gel (figure 3 and 4). The resulting RMSEP values of the three validation sets were 0.0255, 0.0235 and 0.0381 % (w/w). The PLS model of the suspension, measured with the Raman PhAT probe, was constructed using the same strategy as described above and detailed information regarding the construction of the model together with the resulting RMSECV and RMSEP values can be found in table 1 and 2.

3.2. Validation of the calibration models

3.2.1. UV spectroscopy

The accuracy profile for the UV-based in-line quantification method of the gel is displayed in figure 5a. At each validation concentration level, the β-expectation tolerance intervals exceeded the acceptance limits (10 % relative error) (figure 5a). Furthermore, the predictions of the lowest API concentration level (1.75 % w/w) were more biased than the other concentration levels (table 3). This is probably because of the difficulty to detect this low API concentration. The calculated precision parameters (repeatability and intermediate precision) from the UV-based in-line quantification method showed that the intermediate precision Relative Standard Deviation (RSD) was much higher compared to the repeatability RSD at all concentration levels (table 3). Because of the lower intermediate precision, an important day or operator effect was causing variability in the predictions.

The accuracy profile of the UV-based in-line quantification method of the suspension is displayed in figure 6a. Between the API concentration range of 0.0865 - 0.0955 % (w/w),

the β-expectation tolerance intervals fell within the acceptance limits of 10 % (relative error). Therefore, future measurements between an API concentration of 0.0865 and 0.0955 % (w/w) obtained by this procedure have a probability of 95 % that the difference between the measured concentration and the true concentration is less than 10 % (relative error). However, the β-expectation tolerance intervals at the lowest (0.0774 % w/w) and highest (0.1046 % w/w) API concentration level were almost exceeding the 20 % (relative error) acceptance limits. The relative bias at API concentration level 0.0774 and 0.1046 % (w/w) was 3.04 and -4.05 %, respectively. This value is remarkably higher than the relative bias (1.40, 0.65 and -0.92 %) of the other validation concentration levels. Furthermore, a higher imprecision for the lowest and highest concentration level was observed, which was mainly induced by a low intermediate precision, suggesting an important day or operator effect. Table 2 shows that the RMSEP of day 1 (0.00496 % w/w) was almost four times higher than the RMSEP of day 2 (0.00148 % w/w) and 3 (0.00171 % w/w). A cause for the less accurate predictions of the day 1 validation samples was not found, but could be operator related such as an accidental alteration in the production process of these validation samples.

3.2.2. Raman spectroscopy

For the accuracy profile of the Raman-based in-line quantification method of the gel, the β-expectation tolerance intervals exceeded the 10 % (relative error) acceptance limits only at the 1.75 % (w/w) API concentration level (figure 5b). Hence, in the 1.96 - 2.37 % (w/w) API concentration range, 95 out of 100 future measurements will be included within the acceptance limits of 10 % (relative error) and even within the 5 % (relative error) acceptance limits, when using this analytical method. To explain the large β-expectation

tolerance interval at the 1.75 % (w/w) API concentration level, the trueness and precision were investigated. The calculated relative bias and RSD for repeatability at this level were not higher than for the other concentration levels, but the intermediate precision RSD was higher (table 3). There was indeed one validation batch (day 3) where the predictions of the lowest concentration level were lower in comparison to the other validation batches. This variability could be caused by the detection sensitivity limitations of the Raman method at the lowest concentration level.

The accuracy profile for the in-line Raman-based quantification method of the suspension was developed following the same strategy as described above and is displayed in figure 6b. The β-expectation tolerance intervals exceeded the 10 % (relative error) acceptance limits over the whole concentration range, except for the API concentration levels 0.0862 and 0.0953 % (w/w). The accuracy profile has a clear downward trend, i.e., low concentration levels were predicted higher, the intermediate concentration level was predicted around the target concentration and the high concentration levels were predicted lower. This demonstrated that all the concentration levels were predicted as the same value, indicating that the small changes in API concentration could not be detected and that the quantification of the low-dosed API in this suspension could not be achieved with Raman spectroscopy.

When the accuracy profiles and validation parameters of the UV and Raman quantification methods of the suspension are compared, it is clear that the in-line quantification of the API only was possible with UV spectroscopy (table 2 and 3). To better understand the difference in predictive performance of both spectroscopic techniques, the in-line UV and Raman spectra of the suspension calibration set were investigated (figure 7). The UV

spectra are clearly separated according to API concentration between 310 - 325 nm, which confirmed the quantification ability and high sensitivity of UV spectroscopy for this API. In the Raman spectra, no spectral differences between the concentration levels are seen and no API specific peaks can be located in the spectra of the suspension, despite investigating a region of the spectra where the API is Raman active. Increasing the exposure time and number of scans of the Raman spectrometer had no impact on the detection of the API.

The high sensitivity of UV spectroscopy was correlated with the strong UV activity of the API in the suspension, due to conjugated double bonds in its molecular structure [38], [40]. However, the molecular structure of the API also meets to the requirements (non-polar bonds and aromatic rings) for good Raman activity, suggesting that the failure of the Raman method for the suspension is linked to the inherent weak Raman effect [17], [41]. Raman spectroscopy applies monochromatic light to irradiate the samples and the incident light is scattered by the sample molecules. Most of this light is scattered at the same frequency, i.e., Raleigh radiation. Only one in 10^8 incident photons is scattered with a different frequency than the incident light (Raman effect). This in combination with the small fraction of light which is scattered into the same direction of the probe, explains why the quantification of low concentrations can be an issue for Raman spectroscopy [41].

UV spectroscopy was identified as a novel and alternative in-line spectroscopic tool for quantification purposes, in addition to the widely used Raman spectroscopy. Important is that none of the two spectroscopic techniques was superior to the other for both the formulations. While Raman was more accurate in quantifying the API in the gel (2 % w/w), the in-line UV-based method for the suspension performed better than the in-line Raman-

based method. This study illustrated that spectroscopic techniques can be complementary and that the preferred technique is dependent on several factors such as the molecular structure of the API, concentration of the analyte, measurement conditions, presence of interfering components, measurement time and cost. In addition, the UV immersion probe was more practical to work with inside a process environment, because the probe tip can be in direct contact with the sample. Furthermore, UV spectroscopy is a suitable PAT-tool for measurements in aqueous environments, since the suspension contained water. This would be challenging for NIR spectroscopy because water creates strong absorbance peaks in the near infrared region, which can potentially overwhelm the signal(s) of the API [41]. Preliminary off-line experiments with NIR spectroscopy showed that the APIs had weak signals in the near infrared region and therefore NIR spectroscopy was not further investigated in this study.

The measurement uncertainty of the UV- and Raman-based calibration models is summarized in table 4 in terms of the uncertainty of the bias, uncertainty, expanded uncertainty and the relative expanded uncertainty at each concentration level of the validation sets [36]. For the UV-based method of the suspension, the relative expanded uncertainty at the target API concentration (0.09 % w/w) was 3.82 % (relative error) (table 4). This means that the unknown true value is located at a maximum of ± 3.82 % (relative error) around the measured value, with a confidence level of 95 %. In comparison, the relative expanded uncertainty at the target concentration of the suspension was 6.53 % (relative error) for the Raman-based method.

4. CONCLUSIONS

In this study, analytical methods based on in-line UV spectroscopy were developed for the quantification of APIs in pharmaceutical semi-solid and liquid formulations. The performance of this new PAT-tool was compared with an already more established PAT-method based on Raman spectroscopy. In-line UV measurements were carried out with an immersion probe while for the Raman measurements a PhAT probe was used. The validation of the analytical methods was evaluated by the calculation of accuracy profiles, ensuring that 95 out of 100 future routine measurements will be included within the present acceptance limits of 10 % (relative error). Furthermore, the uncertainty of bias and the expanded uncertainty were estimated at each concentration level. The results show that the calibration model developed from the Raman PhAT probe data had a higher accuracy than the UV-based model for the gel formulation (2 % (w/w) API). The UV method developed for the low-dosed suspension (0.09 % (w/w) API) had good performance characteristics, whereas the quantification of this low concentration was not possible with Raman spectroscopy due to detection sensitivity limitations. It was demonstrated that UV spectroscopy can be adopted as a novel PAT-tool for in-line and real-time quantification purposes during the manufacturing of pharmaceutical semi-solid and liquid formulations and that it can be complementary to other spectroscopic techniques, especially when the detection sensitivity is not sufficient. However, the feasibility of the spectroscopic technique is case dependent and should therefore be assessed in preliminary feasibility studies.

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	Gel (2 % w/w)		Suspension (0.09 % w/w)	
	UV	**Raman**	**UV**	**Raman**
Exposure time (s)	1	15	0.95	15
Scans	5	1	5	1
Preprocessing methods	Mean-centering SNV 1st derivative	Mean-centering SNV 1st derivative	Mean-centering 1st derivative	Mean-centering SNV 1st derivative
Spectral region (UV: nm, Raman: cm^{-1})	280.1-296.9	390.1-404.8 655.0-666.7 1340.2-1354.9 1570.0-1600.0	310.1-325.6	1390.0-1430.2
R^2	0.988	0.973	0.995	0.115
Q^2	0.988	0.973	0.995	0.028
# of PLS components	2	1	2	1

Table 1. Exposure time, number of scans, preprocessing methods, spectral region(s), R^2, Q^2 and number of PLS components of the developed calibration models.

	Gel (2 % w/w)		Suspension (0.09 % w/w)	
	UV	**Raman**	**UV**	**Raman**
RMSECV (% w/w)	0.0274	0.0418	0.000819	0.0108
RMSEP day 1 (% w/w)	0.0584	0.0255	0.00496	0.00947
RMSEP day 2 (% w/w)	0.0709	0.0235	0.00148	0.00996
RMSEP day 3 (% w/w)	0.0588	0.0381	0.00171	0.00951

Table 2. RMSECV and RMSEP values of the UV and Raman calibration models for each formulation.

Spectroscopic technique	Concentration level (% w/w)	Relative bias (%)	Repeatability (RSD, %)	Intermediate precision (RSD, %)	Relative B-expectation tolerance limits (%)
Gel (2 % w/w)					
UV	1.75	-3.02	1.239	2.341	-11.14;5.10
	1.96	-0.88	0.838	2.652	-13.82;12.05
	2.06	-0.01	0.496	2.465	-12.21;12.19
	2.16	0.12	1.869	3.277	-11.56;11.80
	2.37	-1.05	1.040	2.226	-11.77;9.66
Raman	1.75	0.17	0.815	2.097	-10.12;10.46
	1.96	-0.48	1.127	1.225	-3.46;2.49
	2.06	-1.13	0.881	0.944	-3.36;1.10
	2.16	-0.97	0.627	0.815	-3.23;1.29
	2.37	-0.89	0.726	1.051	-4.07;2.30
Suspension (0.09 % w/w)					
UV	0.0774	3.04	0.752	4.479	-19.83;25.91
	0.0865	1.40	0.411	1.376	-5.47;8.28
	0.0910	0.65	0.417	1.653	-7.57;8.86
	0.0955	-0.92	0.508	1.848	-9.95;8.11
	0.1046	-4.05	0.785	4.501	-25.44;17.35
Raman	0.0772	16.06	2.425	3.035	6.72;25.40
	0.0862	4.90	1.437	1.437	1.40;8.40
	0.0908	0.28	1.653	2.910	-10.11;10.67
	0.0953	-4.08	2.429	2.429	-9.49;1.32
	0.1044	-12.65	2.744	2.744	-18.21;-7.09

Table 3. In-line UV and Raman quantification methods: validation parameters per concentration level for the gel and suspension.

Spectroscopic technique	Concentration level (% w/w)	Uncertainty of the bias (% w/w)	Uncertainty (% w/w)	Expanded uncertainty (% w/w)	Relative expanded uncertainty (%)
Gel (2 % w/w)					
UV	1.75	0.0204	0.0447	0.0894	5.10
	1.96	0.0286	0.0588	0.1177	6.01
	2.06	0.0289	0.0584	0.1168	5.67
	2.16	0.0356	0.0794	0.1588	7.34
	2.37	0.0275	0.0590	0.1180	4.98
Raman	1.75	0.0200	0.0418	0.0837	4.78
	1.96	0.0083	0.0252	0.0505	2.58
	2.06	0.0065	0.0203	0.0405	1.97
	2.16	0.0075	0.0190	0.0380	1.76
	2.37	0.0114	0.0271	0.0543	2.29
Suspension (0.09 % w/w)					
UV	0.0774	0.0020	0.0041	0.0082	10.63
	0.0865	0.0007	0.0014	0.0028	3.20
	0.0910	0.0009	0.0017	0.0035	3.82
	0.0955	0.0010	0.0020	0.0040	4.20
	0.1046	0.0026	0.0052	0.0104	9.94
Raman	0.0772	0.0011	0.0029	0.0059	7.63
	0.0862	0.0004	0.0014	0.0027	3.14
	0.0908	0.0013	0.0030	0.0059	6.53
	0.0953	0.0006	0.0023	0.0046	4.85
	0.1044	0.0007	0.0026	0.0052	4.99

Table 4. In-line UV and Raman quantification methods: estimates of the measurement uncertainties on the API concentration at each concentration level per formulation.

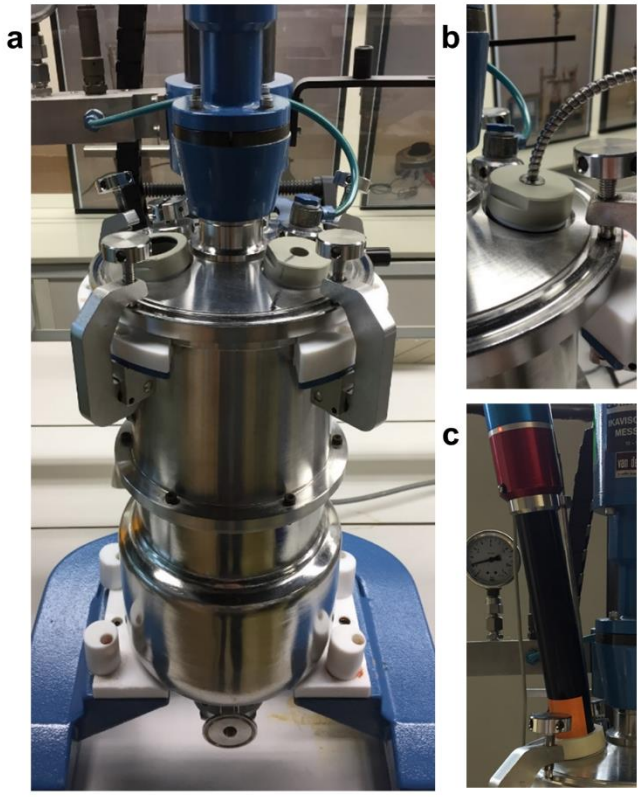

Figure 1. Experimental setup: (a) customized mixing system without probes; (b) UV immersion probe; (c) Raman PhAT probe.

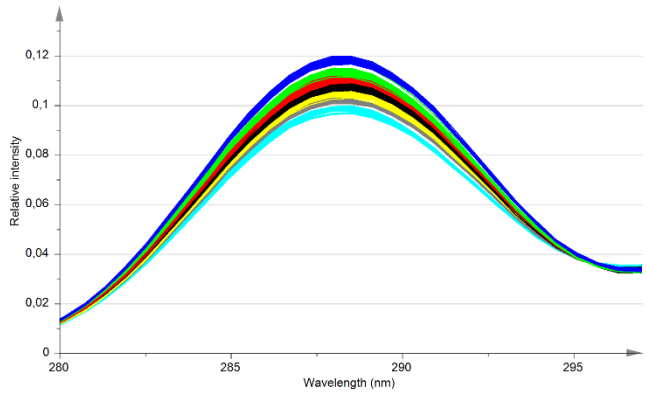

Figure 2. In-line UV spectra of the gel calibration batch between 280 - 300 nm (SNV and first derivative). Turquoise: 80 %, grey: 90 %, yellow: 95 %, black: 100 %, red: 105 %, green: 110 %, blue: 120 %.

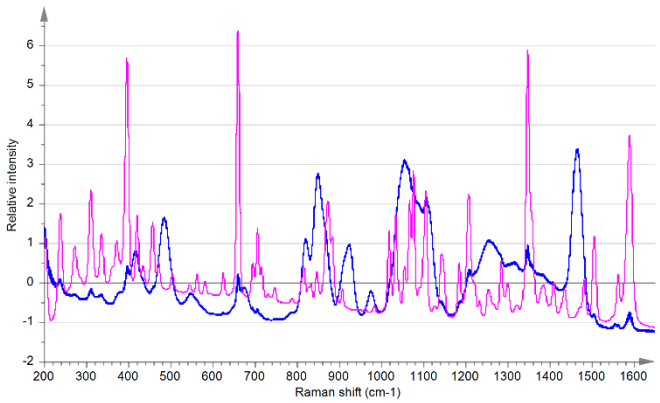

Figure 3. In-line Raman spectra (SNV) of the gel calibration batch (blue) and off-line spectra of the pure API (pink).

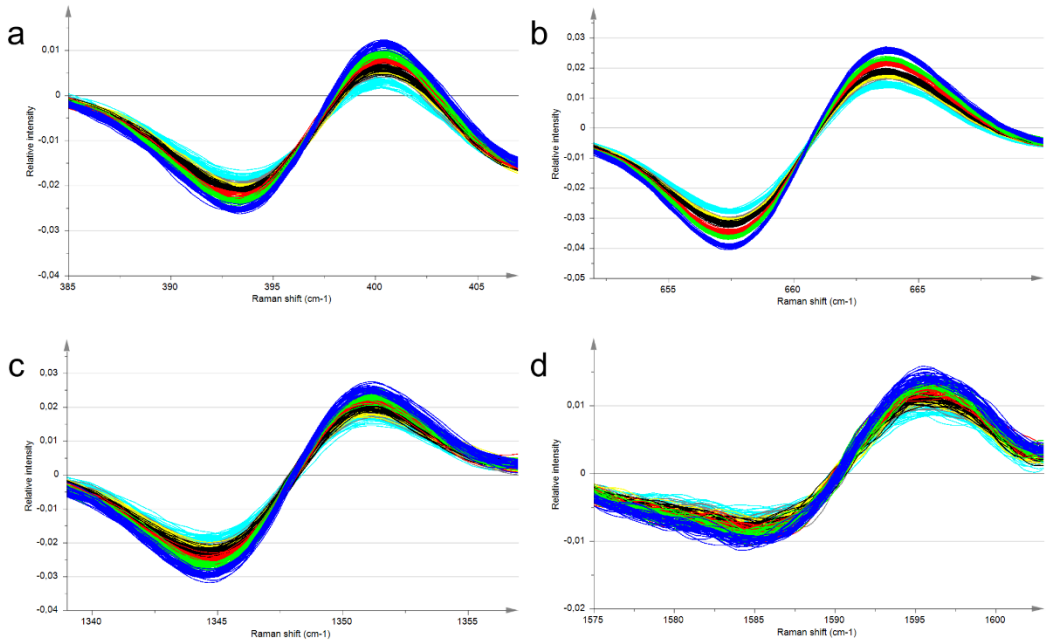

Figure 4. Detail of in-line Raman spectra (SNV and first derivative) of the gel calibration batch at the following spectral regions : (a) 385 – 407 cm^{-1}, (b) 652 – 669 cm^{-1}, (c) 1339 – 1357 cm^{-1} and (d) 1575 – 1602 cm^{-1}. Turquoise: 80 %, grey: 90 %, yellow: 95 %, black: 100 %, red: 105 %, green: 110 %, blue: 120 %.

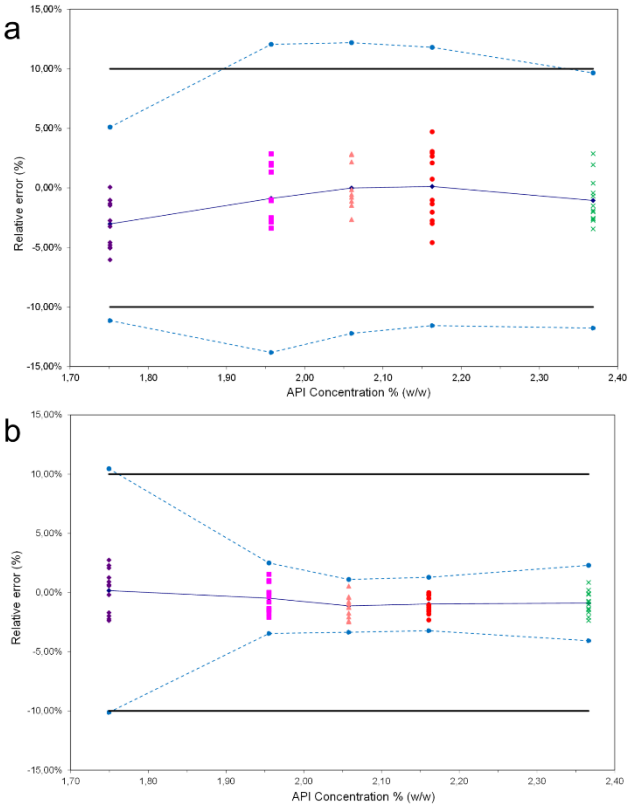

Figure 5. Accuracy profiles of the (a) UV and (b) Raman in-line quantification methods for the gel. Plain black lines: acceptance limits set at 10 % (relative error), dashed blue lines: β-expectation tolerance limits, plain blue line: relative bias.

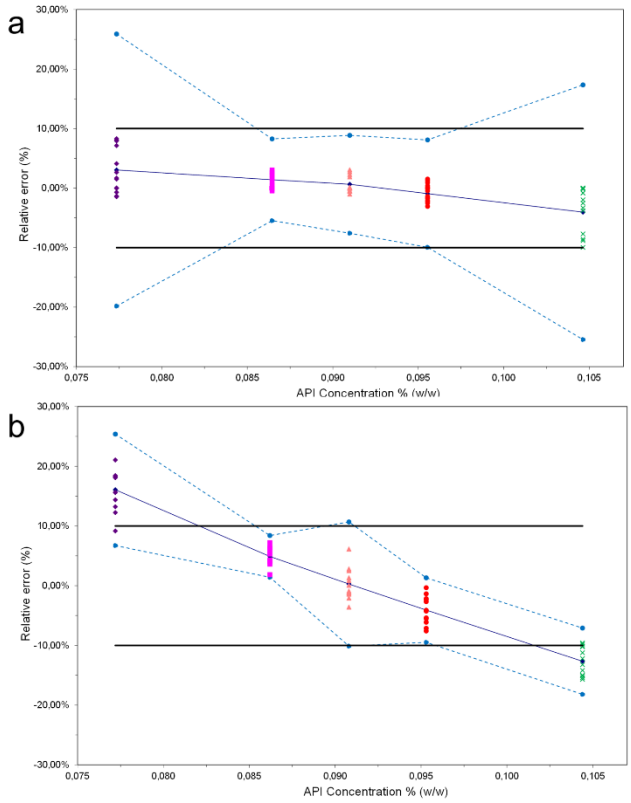

Figure 6. Accuracy profiles of the (a) UV and (b) Raman in-line quantification methods for the suspension. Plain black lines: acceptance limits set at 10 % (relative error), dashed blue lines: β-expectation tolerance limits, plain blue line: relative bias.

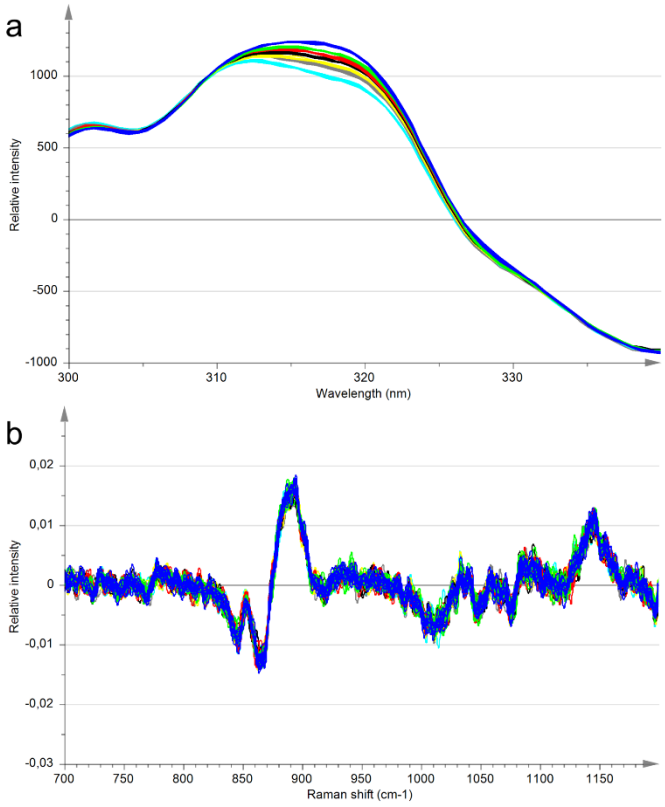

Figure 7. Preprocessed in-line (a) UV and (b) Raman spectra of the suspension calibration batch. Turquoise: 80 %, grey: 90 %, yellow: 95 %, black: 100 %, red: 105 %, green: 110 %, blue: 120 %.